published LTRPC2/TrpC7 sequence. This was interpreted as a possible polymorphic form of LTRPC2/TprC7, therefore an otherwise identical "wild type" LTRPC2 expression construct was also produced. FLAG-LTRPC2 and FLAG-LTRPC2(S1367G) constructs were used in each of the various types of experiments presented, and were indistinguishable in terms of their biochemical and biophysical behavior.—

On page 45, immediately preceding the claims, please insert the enclosed text entitled "SEQUENCE LISTING".

REMARKS

The specification has been amended to include a Sequence Listing and proper reference to the sequences therein. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Entry of this amendment is respectfully requested. The amendments are made in adherence with 37 C.F.R. § 1.821-1.825. This amendment is accompanied by a floppy disk containing the above named sequence, SEQUENCE ID NUMBERS 1-10 in computer readable form, and a paper copy of the sequence information. The computer readable sequence listing was prepared through use of the software program "PatentIn" provided by the PTO. The information contained in the computer readable disk is identical to that of the paper copy. This amendment

contains no new matter. Applicant submits that this amendment, the accompanying computer readable sequence listing, and the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R. § 1.821-1.825.

Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

DORSEY & WHITNEY LLP

Dated: 6-17-02

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Filed under 37 C.F.R. Section 1.34(a)

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at page 6, line 5, has been amended as follows:

— **Fig. 1** depicts the protein sequence analysis of LTRPC2. Fig. 1(A) is a schematic of LTRPC2 structural motifs based on alignments of various related proteins including MLSN-1, LTRPC7, MTR-1, and the *C. elegans* proteins C05C12.3, T01H8.5, and F54D1.5. Bottom: ClustalW alignment of the NUDT9 homology region of LTRPC2 (positions 1197 to 1503 of SEQ ID NO:1), EEED8.8 (SEQ ID NO:4), and NUDT9 (SEQ ID NO:5). The putative signal peptide or anchor found in NUDT9 is double underlined (prediction based on SignalP2.0 analysis of the NUDT9 amino acid sequence). The Nudix box region is boxed by thick lines. Fig. 1(B) shows a qualitative RT-PCR analysis of LTRPC2 and NUDT9 expression in a selection of human tissues. Primers specific for either LTRPC2 (138 bp band) or NUDT9 (252 bp band) were used to prime PCR reactions from cDNA libraries prepared from the indicated tissues. A lack of band of the correct size was interpreted as negative (-), and the presence of a band was interpreted as positive (+). A 4.0 kb partial LTRPC2 cDNA (including the 5' end, and terminating at the internal NotI site) was subsequently cloned from the same leukocyte cDNA library used for these PCR reactions. Multiple NUDT9 cDNAs were obtained from a single screening of the same spleen cDNA library used for these PCR reactions.—

Paragraph beginning at page 38, line 13, has been amended as follows:

— **Example 1: RT-PCR and northern blot analysis of expression.** For PCR analysis of LTRPC2 expression, the oligos used were CAGTGTGGCTACACGCATGA (SEQ ID NO:6) and TCAGGCCCGTGAAGACGATG (SEQ ID NO:7) to produce a 138 bp band. For analysis of NUDT9 expression, the oligos used were GGCAAGACTATAAGCCTGTG (SEQ ID NO:8) and ATAATGGGATCTGCAGCGTG (SEQ ID NO:9) to produce a 252 base pair band. Amplification conditions used were 95 degree melting, 55 degree annealing, and 72 degree extension for 25 cycles. All libraries screened were from Life Technologies. For northern blots, single stranded probes were constructed with the NotI/BglII fragment of the human LTRPC2 sequence as template using an Ambion StripEZ T7 RNA probe kit according to the manufacturers instructions. RNA was extracted from the indicated cell lines using the FastTrack mRNA extraction kit (Invitrogen), and transferred to nylon membranes using standard methods. Hybridizations were performed using Ultrahyb hybridization buffer (Ambion) at 65-68 degrees and otherwise standard methods.—

Paragraph beginning at page 39, line 10, has been amended as follows:

— **Example 3: Construction of a FLAG-tagged LTRPC2 expression construct.** Brain cDNA was purchased from Clontech and used to obtain by RT-PCR the LTRPC2 coding sequence not present in the 4.0 kb fragment isolated by cDNA cloning. This sequence extended from the internal NotI site present in LTRPC2 to the stop codon, and included an additional KpnI site just internal to the stop codon, thereby adding an additional two amino acids (glycine and

threonine) to the 3' end of LTRPC2, followed by a stop codon and a SpeI site just beyond the stop codon. This RT-PCR fragment was ligated onto the 4.0 Kb cDNA using the NotI site and SpeI sites, producing a full length LTRPC2 coding sequence. The internal NotI site in this full-length LTRPC2 template was then removed by site-directed mutagenesis, and PCR was used to generate a LTRPC2 expression construct containing a NotI site at the 5' end internal to the initiating methionine. This construct was subcloned into a modified pCDNA4/TO vector containing a Kozak sequence, initiating methionine, FLAG tag, and polylinker including a NotI site in appropriate frame with the FLAG tag and a 3' SpeI site. This produced an expression plasmid that yielded a protein with the following predicted sequence: MGDYKDDDDKRPLA- (SEQ ID NO:10) followed by the LTRPC2 coding sequence beginning at amino acid 3 and extending to amino acid 1503- followed by GT and then the stop codon. Sequencing of the full-length LTRPC2 construct showed four single base pair differences with the original LTRPC2/TrpC7 sequence. Three of these did not change the predicted amino acid sequence, while the fourth introduced a glycine for serine substitution at amino acid 1367 relative to the published LTRPC2/TrpC7 sequence. This was interpreted as a possible polymorphic form of LTRPC2/TprC7, therefore an otherwise identical "wild type" LTRPC2 expression construct was also produced. FLAG-LTRPC2 and FLAG-LTRPC2(S1367G) constructs were used in each of the various types of experiments presented, and were indistinguishable in terms of their biochemical and biophysical behavior.—

On page 45, immediately preceding the claims, the enclosed text entitled "Sequence Listing" was inserted into the specification.